{Exhibit 35}

Langer and Ward, Abstract 1153: "A Rapid and Sensitive Immunological Method for In Situ Gene Mapping in Journal of Supramolecular Structure and Cellular Biology, (1981)

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A RAPID AND SENSITIVE IMMUNOLOGICAL METHOD FOR IN SITU GENE MAPPING. Pennina R. Langer and David C. Ward, Department of Human Genetics, Yale University, New Haven, CT 06510
A method for in situ localization of specific DNA sequences has been developed which exploits the interaction between modified nucleotides and antibodies directed agianst the modification. Cloned, unique sequences of drosophila DNA are nick translated in vitro in the presence of E. Coli DNA Polymerase I and an analogue of dUTP which contains a biotin molecule covalently linked to the C_5 position of the pyrimidine ring. The nick translated probe containing approximately 0.25% biotin substituted nucleotides, is hybridized in situ, according to standard protocols, to drosophila salivary gland chromosomes. After hybridization, the slides are incubated with monospecific Rabbit antibiotin followed by FITC-Goat anti-rabbit IgG. After counterstaining with Evan's Blue, a single yellow-green fluorescent band, corresponding to the map location of the cloned DNA is seen against a red fluorescent background of the salivary gland chromosomes. Current work is aimed at further refining the system for use in localization of unique sequences on mammalian metaphase chromosomes. FITC-avidin and histochemical reagents are under investigation as substitutes for the indirect immunofluorescent localization of hybridized regions. Other potential applications of biotinyl polynucleotides as affinity reagents will also be presented.

1154 DEVELOPMENTAL REGULATION OF SEA URCHIN HISTONE GENES IN XENOPUS EGGS AND OOCYTES, Laurence Etkin, Dept. of Zoology, University of Tennessee, Knoxville, TN 37916
Sea urchin histone genes H1, H4, and H2B, contained in the recombinant plasmid pSpl02, were microinjected into either the germinal vesicle of oocytes or the cytoplasm of fertilized eggs of Xenopus laevis. Recipient oocytes were treated with progesterone, to induce maturation, and incubated in ^{3}H lysine to label newly synthesized histones. Histone proteins were extracted with 10% GuCl-40% ETOH and analyzed by two dimensional gel electrophoresis and fluorography. Newly synthesized sea urchin H1, and H2B histones were detected in recipient oocytes cultured without progesterone. These proteins are not observed, however, in recipient oocytes which have matured in response to progesterone. This suggests that the sea urchin histone genes are not expressed in oocytes undergoing maturation. When the plasmid pSpl02 is injected into fertilized eggs shortly after artificial insemination, sea urchin histones are not detected until the late gastrula- early neurula stage of development, and continue to be expressed as late as the tailbud stage. These results indicate that the microinjected sea urchin histone genes are expressed concurrently with endogenous Xenopus histone genes in growing oocytes, matured oocytes, and embryos, suggesting that the sea urchin genes are able to recognize and respond to endogenous Xenopus gene regulatory signals.

1155 USE OF RECOMBINANT DNA MOLECULES TO INVESTIGATE REGULATORY SIGNALS FOR THE INITIATION OF TRANSCRIPTION, Peter Gruss and George Khoury, National Institutes of Health, Bethesda, MD 20205
Nucleotide signals required for the initiation of transcription for the early region of the SV40 genome have been identified. This has been achieved by deleting portions of the genome between 0.69 and 0.71 map unit. A 72 bp repeat has been found to harbor a cis essential function, which cannot be provided in trans by either the early tsA28 or the late tsB4 mutant. Further studies have shown that this mutant is incapable of inducing early viral RNA as examined by S1 nuclease analysis, or the early gene product, T-antigen. Studies are in progress to determine whether this mutant can replicate its DNA in the presence of exogenously provided T-antigen. Further attempts to characterize this repetitive element involve replacing the SV40 72 bp repeats by a related repeat unit derived from Moloney sarcoma virus. Results of these experiments will be discussed in detail.